PCT/NZ00/00116

10/019506

RECD 24 JUL 2000
WIPO PCT

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CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 29 June 1999 with an application for Letters Patent number 336505 made by DIATRANZ LTD.

Dated 4 July 2000.

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17 1(a) OR (b)

Neville Harris
Commissioner of Patents

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Patents Form 4.

Patents Act 1953

Provisional Specification.

Anti-diabetogenic milk or milk products.

We, **Diatranz Ltd,** a company incorporated in **New Zealand,** *of* **19 Laureston Avenue, Otahuhu, New Zealand,**

do hereby declare this invention to be described in the following statement:

TITLE ANTI-DIABETOGENIC MILK OR MILK PRODUCTS

FIELD

This invention relates to the disease Type 1 diabetes (insulin-dependent diabetes mellitus or IDDM) and to products and methods for its control; in particular this invention relates to one or more ingestable materials (initially of dairy origin) capable of reducing the incidence of diabetes in a population. More particularly, said ingestable material, after proteolysis, has a peptide nature and is capable of acting as an immuno-modulating agent.

BACKGROUND

Diabetes is a not uncommon disease affecting carbohydrate metabolism and glucose levels in the blood. The disease has several forms. Type 1 diabetes (insulin-dependent diabetes mellitus or IDDM) is a type of autoimmune disease in which antibodies to islet cells appear and later the production of endogenous insulin is prevented, requiring exogenous insulin treatment for the remainder of the patient's life. This specification relates to Type 1 diabetes in particular.

During digestion (proteolysis) in the gut, some isomers of β-casein (A1 or B for example) from cow's milk may undergo only partial proteolysis so that small peptides such as β-casomorphin 7 derived from bovine β-casein A1 make their way into the circulation. Over the last 15 years or so, a number of publications have considered the numerical association for a number of countries between ingestion of cows' milk or

products thereof and Type 1 diabetes in juveniles (as well as some other diseases) but not until critical analysis by the inventor, published as Elliott RB et al (Diabetologia (1999) 42: 292-296) has a clear correlation between the two been identified. This paper provides for the apparent anomaly of Icelandic statistics (high dairy foods consumption; low incidence). This paper found a strong correlation between the incidence of Type 1 diabetes and a weighted average of consumption of β-casein types A1 and B (that is, excluding type A2).

Peptides having a structure in which proline residues alternate with any amino acid tend to be resistant to digestion by endoproteases in the gut, and may be left intact after digestion of proteins containing them. Casomorphins have that structure. Variants A1 and B of bovine β-casein yield some β-casomorphin 7 (PRO-GLY-PRO-ILE-PRO-GLY) from residues (for type A1) 63 to 68 inclusive, after proteolysis in the gut. (Teschemacher (US 4681871) teaches the isolation and use of various orally active casomorphins, preferably short peptides having opiate like or analgesic activity, such as β-casomorphin 3 for use in analgesia, but makes no reference to type A2 casein nor to functional relationships of casomorphins with diabetes). Type A2 β -casein does not yield β-casomorphin 7 owing to a further proline residue at site 67 in that casein. β-casomorphin 7 has opioid-like properties including some action on the gut itself such as motility, absorbtion, and secretion. It is a direct inhibitor of acetyl-choline esterase.

There is strong circumstantial evidence as revealed by Elliott et al (1999) that the incidence of diabetes is closely related to the quantity of dairy products consumed and in particular to the amount of β-casein A1.

The patent application WO96/14577 (inventors Elliott & Hill; priority date 4 November 1994) teaches that milk protein genes are expressed in a codominant way, so that individual phenotypes typically result in mixed caseins such as A1A2, A2A3, A2B, and so on. Gene frequencies vary between breeds. The application teaches that for use in dairy production selection of only those cows that have an A2A2 genotype

and produce an alternative variant of casein, β-casein A2, (not A1 nor A1A2 nor B) or alternatively the consumption of dairy products not containing β-casein A1 will tend to reduce the incidence of diabetes. This specification is based on the appreciation that β-casomorphin 7 apparently tends to cause diabetes.

In other words the presumption has been made in the prior art that the diabetogenic effect of milk including β-casein A1 (or the like) is solely a result of the β-casomorphin 7 inevitably released during proteolysis in the gut. (The thread of this argument is continued below, under "Preferred Embodiment").

OBJECT

It is an object of this invention to provide means for providing at least a partial resistance to type I diabetes, or at least to provide the public with a useful choice.

STATEMENT OF INVENTION

In a first broad aspect this invention provides a product capable of reducing the incidence of Type I diabetes: wherein the product includes a relatively stable compound capable of promoting immunity against diabetes by means of an action occurring in or about the wall of the gut.

More specifically the compound is a peptide and more specifically it includes from seven to twelve amino acid residues.

Typically, proline makes up a large proportion of the residues and preferably every alternate residue is a proline molecule.

Optionally the compound is provided with one or more enhancing agents capable of increasing the effectiveness of the promotion of protection against Type I diabetes.

Preferably the compound is provided as a portion of a more complex molecule comprising part of a foodstuff.

A preferred complex molecule is the protein known as bovine β-casein A2.

Alternative caseins include caseins derived from goat milk, human milk, or the like.

Alternatively the compound may be provided as a peptide, or as pharmaceutically acceptable salts thereof or as pharmaceutically acceptable esters thereof.

In a second broad aspect this invention provides a method for the minimisation of Type I diabetes by providing that those individuals believed to be susceptible to Type I diabetes shall have the opportunity to consume only those dairy products obtained from breeds or strains of dairy animal that produce β-casein A2 and (incidentally) substantially no β-casein A1 nor β-casein B so that the individuals become protected by exposure to a therapeutically effective amount of β-casomorphin 9.

In a third broad aspect this invention provides a method for the minimisation of Type I diabetes by the oral administration of preparations including added β-casomorphin 9, accompanied by, or with foodstuffs whether of dairy origin or not.

Preferably, according to this aspect, food materials may be provided for the purpose of minimisation of the incidence of diabetes; said materials including an effective amount of β-casomorphin 9 or precursors thereof.

Optionally, food materials according to this aspect may be sold for the purpose of minimisation of the incidence of diabetes; said materials including an effective amounts of at least one analogue to β-casomorphin 9 or precursors thereof.

In a related aspect, the β-casomorphin 9 or analogues thereof may be made by recombinant means, or from casein by proteolysis,. or be synthesised.

In a fourth broad aspect this invention provides a method for the minimisation of Type I diabetes by the administration of β-casomorphin 9.

Optionally administration is as a slow-release formulation.

Optionally, administration is as pharmaceutically acceptable salts of β-casomorphin 9 or as pharmaceutically acceptable esters thereof.

In a fifth broad aspect this invention provides a method for the minimisation of Type I diabetes by the administration of "helper materials" which may include;

(a) substances that enhance the enzymic cleavage of β-casomorphin 9 from casein,

(b) substances that enhance the carriage of β-casomorphin 9 into at least the lamina propria of the gut,

(c substances that enhance the response of immunologically capable cells within the body to the existence of β-casomorphin 9.

In a sixth broad aspect this invention provides a method for the minimisation of Type I diabetes by means of employing an improved peptide or the like, having enhanced capabilities in terms of conferring resistance to type I diabetes, over those possessed by β-casomorphin 9.

In a seventh broad aspect this invention provides for the use in a dairy industry, or at least in some commercial aspect thereof, of breeds or strains of dairy animal that produce β-casein A2 and substantially no β-casein A1 nor β-casein B.

Example breeds include the *Bos indicus* subspecies.

Example strains include the dairy cows existing in Iceland (which are a relic population of Norse dairy animals),and/or even humans (that is, avoiding certain types of cows' milk particularly in early life).

Preferably, according to this aspect, foods may be provided for the purpose of minimisation of the incidence of diabetes, wherein the foods are substantially free from the bovine casein variants known as β-casein A1 or β-casein B.

In a further broad aspect this invention provides a method for the creation, by a process of selection from a mixed population of breeds or strains of dairy animal that produce β-casein A2 and substantially no β-casein A1 wherein the method comprises appropriate animal selection methods known in the art, so that only dairy products having substantially only type A2 casein are produced.

In a yet further broad aspect this invention provides a dairy product having undergone purification during a manufacturing process, so as to eliminate β-casein A1 from a product.

PREFERRED EMBODIMENT

The description(s) of the invention to be provided herein is/are given purely by way of example and are not to be taken in any way as limiting the scope or extent of the invention.

DRAWINGS

Fig 1: Graph of results of the BB rat/ Prosobee trial.

Fig 2: Sequence of β-casomorphin 9

Fig 3: Sequence of bovine β-casein A2 showing the position of the sequence for β-casomorphin 9

To continue the thread of the argument from the Background (see above) ... it has since been realised that, while the assertion in relation to the diabetogenic nature of β -casomorphin 7 may be true, there is also experimental evidence for an alternative explanation which should permit more positive manipulation of the autoimmune response resulting in type I diabetes. One characteristic of the casomorphins is that the alternating proline residues will "protect" adjacent peptide bonds from attack by endopeptidases. Note in Fig 3 that there is only the one sustained sequence - that at residue 60 on, although another pro-tyr-pro-glu sequence is at residue 180.

The alternative explanation is based on the discovery that there is a related peptide β -casomorphin 9, including amino acid residues number 60 to number 68 inclusive (See Fig 2) of bovine β-casein A2 (See Fig 3) which peptide is released during the digestion of milk containing that variant. The composition of this peptide also confers resistance against further digestion by endopeptidases. This peptide is believed to have an immunoprotective effect or at least an immunomodulatory effect in relation to Type I diabetes and as a result the consumption of milk including β-casein A2 (and substantially no β-casein A1 nor β-casein B) will result in a reduction in the incidence of diabetes to below the rate of incidence in a control population.

EXAMPLE 1

This study involved "BioBreeding" (BB) rats in a trial. See Fig 1 for a graphical display of the results with diabetes incidence (1.0 = 100%) on the vertical axis.. The control diet was "Prosobee" (TM) which is a soy preparation used as rat food in laboratories. The spontaneous incidence of diabetes in the control population was about 38%. Rats fed "Prosobee" (TM) plus 10% mixed casein (that is, A1 and A2) had an incidence of about 27%. Rats fed on "Prosobee" (TM) plus 10% type A1 casein had an incidence of about 45%. Rats fed on "Prosobee" (TM) plus 10% type A2 casein had an incidence of about 20%. As expected, the incidence of diabetes in the A1 group was higher than that of the control group (which is in accordance with the teaching of WO96/14577).

The inventor's argument is that had the A2 casein (or breakdown products thereof) been simply a "neutral" substance, then in accordance with the teaching of WO96/14577, the incidence of diabetes in the A2 group would be about the same as that of the control group, because the adverse effects of β-casomorphin 7 are absent. In fact the incidence of diabetes in the A2 group was significantly reduced and was the lowest of any group. Therefore the inventor proposes that β-casomorphin 9 exerts a beneficial effect on the incidence of Type I diabetes. Presumably it acts as an immunomodulator. The actual mechanism of action whereby a casein fragment from the milk of one species of animal has an effect on antibodies against β-islet cells of the pancreas in at least humans and susceptible laboratory rodents is unknown but it has been observed that caseins are involved in cellular structures and small peptides such as casomorphins may act as intracellular messengers.

COMMERCIAL APPLICATIONS.

In order to make use of this discovery, many avenues may be explored. For instance...

1. Use, in a dairy industry or for a specific supply of health food materials, of breeds or strains of dairy animal that produce β-casein A2 and substantially no β-casein A1 nor β-casein B. (For example the *Bos indicus* subspecies, and the dairy cows existing in Iceland (which are a relic population of Norse dairy animals), goats, or even humans (where use of the latter may avoid exposure to certain types of cows milk or products thereof particularly in early postnatal life).

2. Selection, from a mixed population, of breeds or strains of dairy animal that produce β-casein A2 and substantially no β-casein A1, A1A2, or B using some or all of the selection methods known in the art. For example, cows may be herd-tested for casein variants secreted and those producing other than the A2 variant rejected. Bulls under consideration as AI sires will be either directly tested using methods of genetic engineering, or daughters (preferably bred from A2 type dams) from the initial proving progeny will be tested as above.

3. Subsequent purification so as to reject β-casein A1 from a product. (This is believed to be relatively infeasible. Casein tends to occur as micelles.).

4. Consumption, at least by individuals known to be susceptible to Type I diabetes, of dairy products only from those breeds or strains of dairy animal that produce β -casein A2 and substantially no β-casein A1 or B.

5. Administration of preparations including β-casomorphin 9 orally, along with foodstuffs whether of dairy type or not. The β-casomorphin 9 may be made by recombinant means, from casein, or may be synthesised.

6. Administration, perhaps in a slow-release formulation so as to promote or extend an immune response of β-casomorphin 9.

7. Administration of "helper materials" which may include...

   (a) substances that enhance the enzymic cleavage of β-casomorphin 9 from proteins containing it,

   (b) substances that enhance the carriage of β-casomorphin 9 into at least the lamina propria of the gut,

   (c) substances that enhance the response of immunologically capable cells within the body to the presence of β-casomorphin 9.

8. Any of the above strategies but instead employing an improved peptide or the like having enhanced capabilities in terms of conferring resistance to type I diabetes over those possessed by β-casomorphin 9. (While β-casomorphin 9 is a naturally occurring peptide having desired activity, further research may lead to more active materials possibly with less adverse effects.

COMMERCIAL BENEFITS or ADVANTAGES

The benefits of this invention include:

(1) A reduction in the national incidence of Type I diabetes would save a good deal of human suffering and would save the nation about one million dollars over the lifetime of each affected person. Because the incidence of Type I diabetes in New Zealand is approximately 18 per hundred thousand person years at risk, it will be appreciated that savings can be made.

(2) Clarification of the role of the variants of casein will allow an improved national herd to be built up by selection based on a rational premise. At the same time, health of the population can be improved without actual medication.

Although various preferred examples as described above have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions, and substitutions are possible without departing from the scope and spirit of the invention as set forth.

Ensor and Associates

for

Diatranz New Zealand Limited

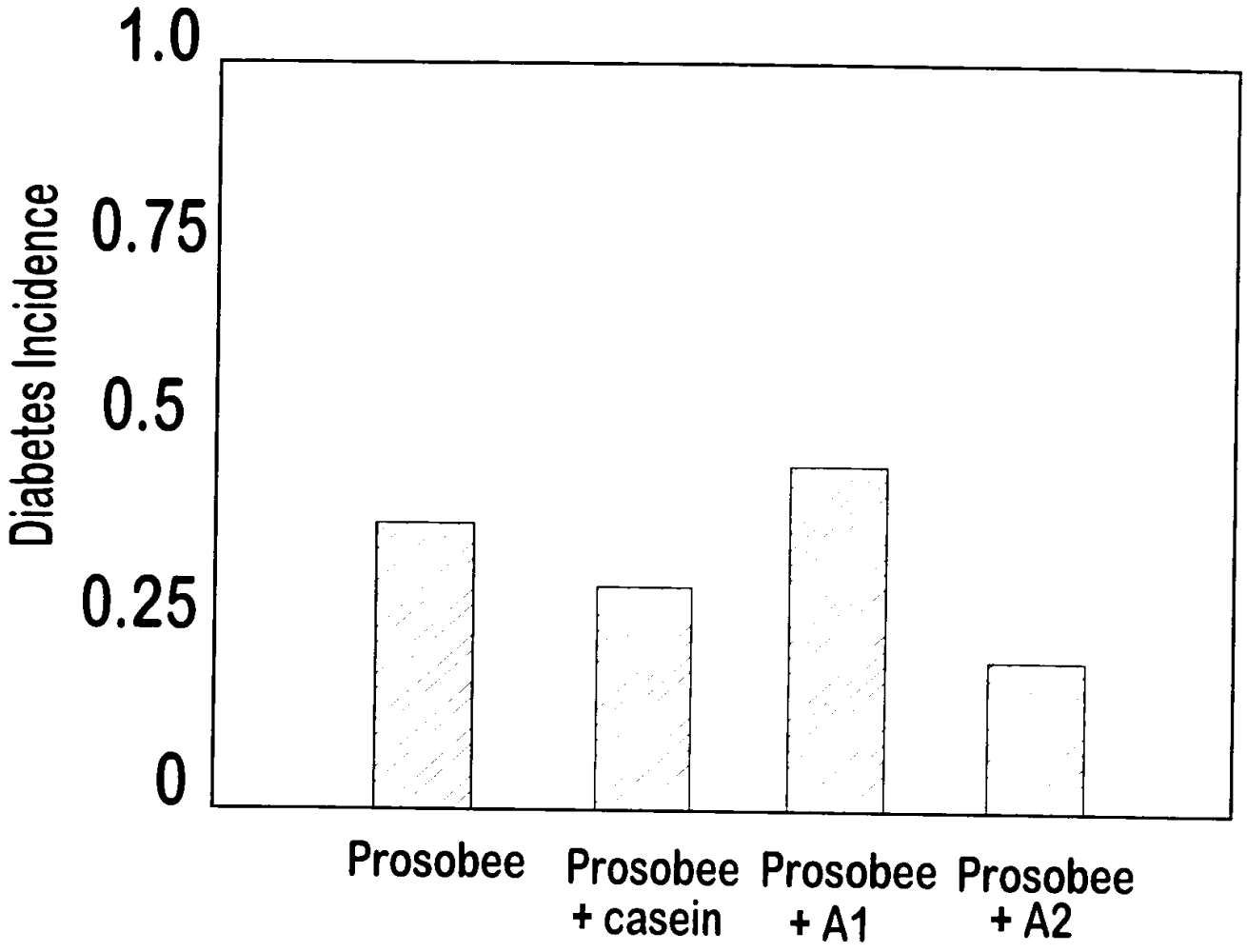
1.0
0.75
0.5
0.25
0
Diabetes Incidence
Prosobee
Prosobee + casein
Prosobee + A1
Prosobee + A2

Fig 1

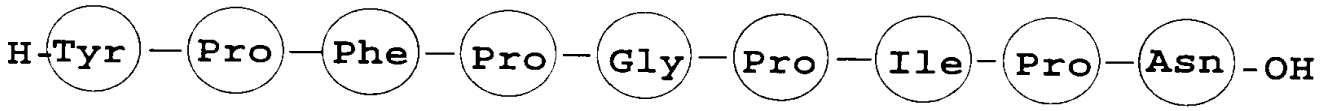
H-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-OH

Fig 2

20

H-Arg-Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Ser-Leu-Ser-Ser-Ser-Glu-

Glu-Ser-Ile-Thr-Arg-Ile-Asn-Lys-Lys-Ile-Glu-Lys-Phe-Glu-Ser-Glu-Glu-Glu-Glu-Glu-

60

Thr-Glu-Asp-Glu-Leu-Glu-Asp-Lys-Ile-His-Pro-Phe-Ala-Glu-Thr-Glu-Ser-Leu-Val-Tyr-

Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-Leu-Pro-Glu-Asn-Ile-Pro-Pro-Leu-Thr-Glu-Thr-

100

Pro-Val-Val-Val-Pro-Pro-Phe-Leu-Glu-Pro-Glu-Val-Met-Gly-Val-Ser-Lys-Val-Lys-Glu-

Ala-Met-Ala-Pro-Lys-His-Lys-Glu-Met-Pro-Phe-Pro-Lys-Tyr-Pro-Val-Glu-Pro-Phe-Thr-

140

Glu-Ser-Glu-Ser-Leu-Thr-Leu-Thr-Asp-Val-Glu-Asn-Leu-His-Leu-Pro-Pro-Leu-Leu-Leu-

Glu-Ser-Trp-Met-His-Glu-Pro-His-Glu-Pro-Leu-Pro-Pro-Thr-Val-Met-Phe-Pro-Pro-Glu-

180

Ser-Val-Leu-Ser-Leu-Ser-Glu-Ser-Lys-Val-Leu-Pro-Val-Pro-Glu-Lys-Ala-Val-Pro-Tyr-

Pro-Glu-Arg-Asp-Met-Pro-Ile-Glu-Ala-Phe-Leu-Leu-Tyr-Glu-Glu-Pro-Val-Leu-Gly-Pro-

209

Val-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val-OH

Bovine beta-casein A2.

Fig 3